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## Review article

# Advanced glycation end products: An emerging biomarker for adverse outcome in patients with peripheral artery disease



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## ABSTRACT

Patients with peripheral artery disease (PAD) suffer from widespread atherosclerosis. Partly due to the growing awareness of cardiovascular disease, the incidence of PAD has increased considerably during the past decade. It is anticipated that algorithms to identify high risk patients for cardiovascular events require being updated, making use of novel biomarkers. Advanced glycation end products (AGEs) are moieties formed non-enzymatically on long-lived proteins under influence of glycemic and oxidative stress reactions. We elaborate about the formation and effects of AGEs, and the methods to measure AGEs. Several studies have been performed with AGEs in PAD. In this review, we evaluate the emerging evidence of AGEs as a clinical biomarker for patients with PAD.

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## 1. Introduction

Peripheral artery disease (PAD) has become a global health problem. Partly because of the growing awareness for cardiovascular disease both among the general public and among health care professionals, the reported prevalence of PAD did increase more than 23% within a decade (2000 until 2010) [1]. Patients with PAD do not only suffer from local symptoms, but they are also at increased risk for cardiovascular events, such as myocardial infarction, stroke and cardiovascular death. Therefore patients with PAD are considered to qualify for secondary cardiovascular prevention.

Within the group of patients with established cardiovascular disease, including PAD, it remains a challenge to identify the very

high risk patient in order to improve stratification for secondary cardiovascular prevention. An important drawback is that the generally proposed cardiovascular risk estimators are designed to identify those patients who classify for primary prevention. In fact, updated algorithms that are employed in the current risk estimators are insufficient for this purpose. The primary prevention Framingham risk score and the Systematic Coronary Risk Evaluation (SCORE) risk chart that are used frequently in the clinic, were developed over 15 years ago [2,3]. In case of secondary cardiovascular prevention, all patients are deemed at high risk, without a generally accepted system for risk differentiation. Due to implementation of cardiovascular preventive medication such as lipid-lowering drugs and antihypertensive drugs as secondary prevention strategies, these established risk factors will be incorporated into risk estimating mostly after being modified. For these reasons, there is a clear need for updated and alternative risk prediction models, especially in secondary prevention, which may also include new risk markers. Therefore, an important challenge of current research in cardiovascular disease, and in PAD in particular, is to focus on finding better ways to predict cardiovascular events. A potential way to achieve this is to search for new biomarkers to identify patients with increased cardiovascular risk.

One group of biomarkers that are increasingly considered to be

Abbreviations: AGEs, advanced glycation end products; ABI, ankle-brachial index; CML, Nε-carboxymethyl-lysine; PAD, peripheral artery disease; ELISA, enzyme-linked immuno sorbent assay; SAF, skin autofluorescence; RAGE, receptor for advanced glycation end products.

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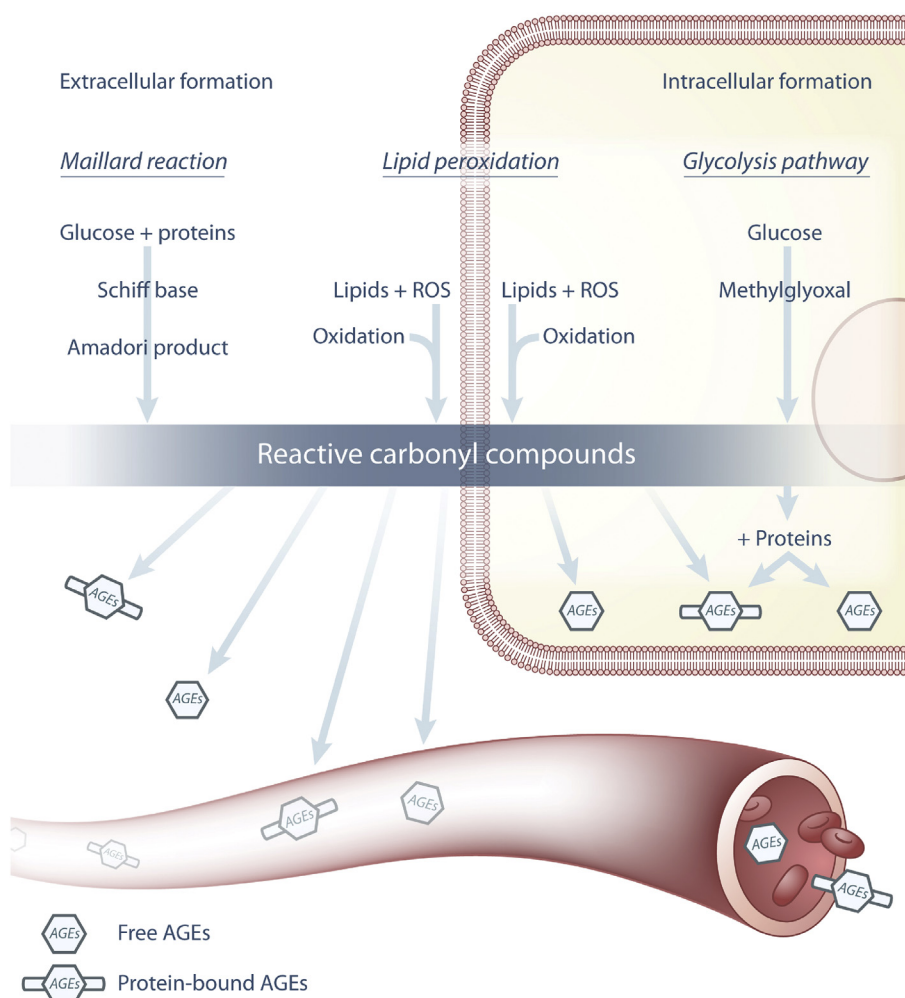
important to better identify high risk patients are advanced glycation end products (AGEs). AGEs are formed by non-enzymatic glycation or by oxidative reactions to form stable structures accumulating on proteins with slow turnover, and are conceivably involved in the process of aging [4]. Negative effects are the formation of cross-links which cause stiffness of interstitial tissue, mitochondrial dysfunction, and binding to the cellular receptors such as receptor for AGE (RAGE) which may lead to cytokine release. These effects modify pathways which contribute to vascular dysfunction and accelerated development of atherosclerotic processes. Increased levels of AGEs have been described in several patient groups at increased cardiovascular risk, specifically those with diabetes mellitus and renal insufficiency [5,6]. In fact, increased AGE levels are associated with cardiovascular endpoints, such as acute myocardial infarction, acute ischemic stroke, and cardiovascular mortality [7–10].

Information on the role of AGEs in patients with PAD is scarce, and this topic has not been reviewed before. In the current review, we evaluate the role of AGEs in PAD. The review first introduces the biochemical background on AGEs, measurement methods, and its association with atherosclerosis. Secondly, we will focus on clinical studies available so far in patients with PAD in which AGEs in plasma, skin and other tissues were measured.

## 2. Accumulation of AGEs

AGEs comprise a heterogeneous group and are formed by a combination of glycation, oxidation, and/or carbonylation, which can be divided into three distinct pathways, as outlined in Fig. 1 [11]. The classical mechanism of AGE formation is the slow Maillard reaction between glucose or reducing sugars and proteins. The interaction between the carbonyl groups of reducing sugars and amino groups of proteins results in the formation of a Schiff base within a few hours. Intramolecular rearrangement of the Schiff base results in more stable Amadori products. Glycated hemoglobin is an example of an Amadori product that is widely used in clinical practice for diagnosis and regulation of diabetes mellitus. The slow process of oxidation of the Amadori products lead to reactive carbonyl compounds and subsequently to the formation of AGEs within weeks to months. The best-known AGEs derived from this glycooxidation process are pentosidine, Nε-carboxymethyl-lysine (CML) and glucosepane [12].

Other, and much faster evolving, processes involving AGE formation are lipid peroxidation and the glycolysis pathway. In the lipid peroxidation pathway, reactive oxygen species (ROS) alter lipids into reactive carbonyl compounds under influence of oxidation. This formation results into AGEs or advanced lipid end products (ALEs), for example malondialdehyde [11]. This reaction takes place both intracellularly and extracellularly.



**Fig. 1. Formation of advanced glycation end products (AGEs).** Formation of AGEs by the three different pathways: Maillard reaction, lipid peroxidation and glycolysis pathway. ROS, reactive oxygen species.

During the intracellular glycolysis pathway, glucose is altered into reactive carbonyl compounds, of which the best-known is methylglyoxal. The chemical reaction between reactive carbonyl compounds and proteins can result in AGEs. An example of AGEs formed by this pathway is methylglyoxal-derived hydroimidazolone (MG-H1).

Besides endogenous formation of AGEs, absorption of exogenous AGEs occurs by two mechanisms. Firstly, accumulation of AGEs occurs by inhalation of tobacco smoke. Tobacco smoke contains highly reactive glycation products which rapidly form AGEs *in vitro* and *in vivo*. Serum AGEs are significantly elevated in smokers who smoke at least a package a day as compared to non-smokers [13]. Secondly, intake of high-AGE food products may lead to an increase of AGEs. The temperature at which food products are prepared is of major importance for the amount of AGEs, with oven frying as most severe inducer [14]. Approximately 10% of the AGEs from food products and beverages are absorbed from the gastrointestinal tract into the blood [15]. For example, serum CML increased after a 6-week high-AGE diet and decreased in low-AGE diet in patients with diabetes mellitus [16].

The final mechanism which affects the exposure to and accumulation of AGEs is the clearance of the kidney and metabolism by the liver. Increased level of AGEs can be found in patients with either renal or liver failure [17,18]. This is also in part attributable to increased production of oxidative stress in these diseases, which stimulates formation of AGEs. However, plasma pentosidine decreased with different types of dialysis in renal failure [17]. Furthermore, plasma pentosidine decreased to 80% six months after renal transplantation, and plasma CML decreased to 50% three years after liver transplantation [17,19]. These findings indicate that the accumulation of AGEs in the blood is at least in part reversible in the context of improvement of kidney and liver function.

### 3. Measurement of AGEs

For appropriate assessment of AGEs levels, the biological sample material as well as the method to measure AGEs are important. Firstly, several methods have been developed to measure AGEs in different body compartments, including blood, urine, and tissue (see Fig. 2). Blood and urine samples can obviously be obtained

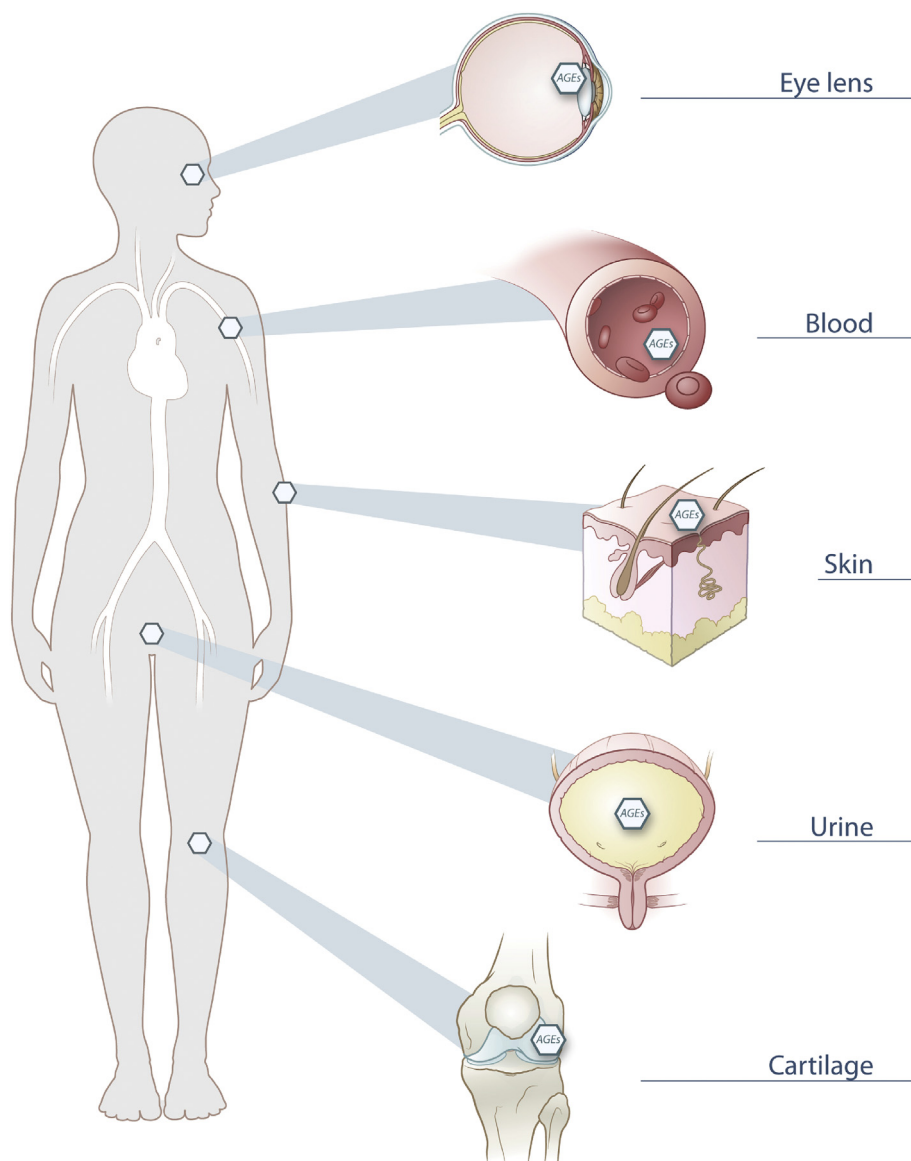


Fig. 2. Measurements of advanced glycation end products (AGEs).

easily; however, it is thought that most AGEs are formed intracellularly, but are also bound intracellularly, or in interstitial tissues. For these reasons, circulating AGEs, especially in plasma, do not sufficiently reflect the AGE amount in tissues [20]. In addition, as stated above, the amount of plasma AGEs is affected by its clearance by kidney and liver. Therefore, concentrations of various AGEs in plasma may fluctuate over time.

The other possibility is to measure AGEs in tissue. An issue of particular importance is the turnover time of tissues. AGEs cross-linked to collagen or other proteins in interstitial compartments are considered to remain linked during the lifetime of the specific tissue. For example, articular cartilage collagen is thought to have an extremely long half-time, which was calculated to be as high as 117 years [21]. AGEs linked to eye lens proteins remain there life-long, and accumulate already from the preconceptual period on. Therefore, these AGEs are considered to represent an estimate of long-term metabolic memory. However, most tissue material is not easy to obtain. A relatively easy tissue to acquire is skin tissue, which was calculated to have a half-life of 14.8 years [21]. For this reason, it has been used in early studies on the role of AGE, for example in patients with type 1 diabetes [22].

Several techniques now available to measure AGEs are described in Table 1 [23]. Traditionally, quantitative measurements of AGEs were performed with enzyme-linked immunosorbent assays (ELISA). However, experts in the field state that for quantitative analysis, ELISA has limited specificity and reproducibility [24]. Furthermore, ELISA kits do not measure the difference between protein-bound and free circulating AGEs. Therefore, studies which use this technique, especially when employing older ELISA kits, should possibly be interpreted with caution. High performance and ultra-high performance liquid chromatography methods, most combined with mass spectrometry, have become the technique of choice to measure both free as well as protein-bound AGEs. A method for localization of AGEs is immunohistochemistry, but this technique is not frequently used.

In addition, the noninvasive method skin autofluorescence (SAF) has been designed to assess the AGEs of the skin with the so-called AGE Reader™ (DiagnOptics Technologies BV, Groningen, the Netherlands). Eye lens autofluorescence has also recently been proposed for assessing lens AGE accumulation as tool for diabetes screening (Clearpath DS, Freedom Meditech, USA). Some AGEs respond to ultraviolet light by emitting fluorescent light with another wavelength. Meerwaldt et al. showed a strong correlation between SAF and the fluorescent AGE pentosidine as well as the

non-fluorescent AGE CML and N $\epsilon$ -carboxyethyl-lysine (CEL) in the dermal layer of the skin [25]. Since the device uses light to detect AGEs, it is difficult to measure patients with a very dark skin, due to absorption of both the incoming light and the fluorescent light. Another limitation of the AGE Reader™ is the effect of skin cream on SAF measurements. Especially self-browning cream and sun blocker cream block the incident light and cause unreliable SAF measurements [26].

#### 4. Vascular effects of AGEs

AGEs are harmful via two main pathogenic pathways (Fig. 3). Firstly, several AGEs have the potential to form cross-links which result in impaired protein function and turnover, and in increased tissue stiffness as a consequence of collagen and elastin cross-linking. Secondly, AGEs may also bind to cell membrane receptors resulting in release of pro-inflammatory cytokines, thereby enhancing inflammatory reactions.

Specific AGEs, such as pentosidine, form cross-links between proteins [27]. Cross-links between and within collagen and elastin fibers result in loss of distensibility and strength, and hence induce arterial stiffness [28]. Results from the Maastricht Study showed a strong association between AGEs and pulse wave velocity as a marker for arterial stiffness. The results showed that plasma protein-bound pentosidine, as well as SAF was positively associated with carotid-femoral pulse wave velocity in 862 patients with normal glucose metabolism, impaired glucose metabolism and type 2 diabetes mellitus [29]. In contrast, plasma CML and CEL were not associated with pulse wave velocity in the latter study. Hofmann et al. showed that tissue AGEs, derived from venous graft material, as well as SAF, were positively associated with pulse wave velocity in patients with coronary artery disease [30]. In addition, stiffness of the heart causes diastolic dysfunction. Several parameter of diastolic dysfunction, measured with echocardiography (e.g. E/A ratio), are associated with both serum AGEs and SAF [31,32].

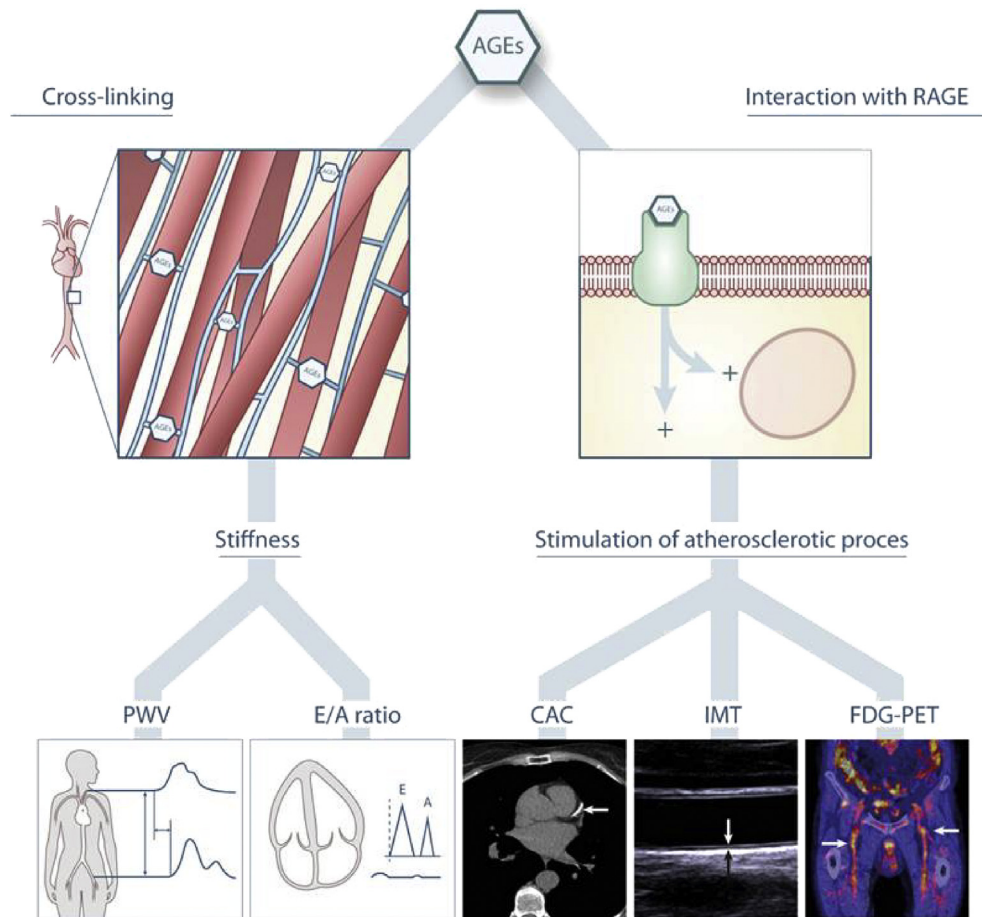
Secondly, AGEs promote cellular stress responses by engagement to receptors on the cell membrane. The binding of AGEs to RAGE results in intracellular activation of nuclear transcription (NF- $\kappa$ B) factor [33]. NF- $\kappa$ B induces the release of several adhesion molecules and pro-inflammatory cytokines such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and interleukin-6. Morita et al. showed that ROS and TNF- $\alpha$  were elevated after stimulation of human aortic endothelial

**Table 1**  
Advantages and disadvantages of techniques to measure AGEs.

Technique	Material	Advantages	Disadvantages
Enzyme-linked immunosorbent assay (ELISA)	Liquids including blood and urine and tissue	- Suitable for large number of measurements	- Limited specificity, and reproducibility - No differentiation between protein-bound and free circulating AGEs - Lack of standard ELISA kits for AGEs
(Ultra-)High performance liquid chromatography	Liquids and tissue	- Accurate and precise measurements - Both free as well as protein-bound AGEs - Measurement of specific AGEs	- Expensive due to specific equipment and dedicated personnel
Immunohistochemistry	Tissue	- Localization of AGEs in tissue	- Invasive biopsies needed before analysis - Lack of standardized antibodies for AGEs
Skin autofluorescence	Skin	- Noninvasive - Fast technique (within seconds) - Point of care testing	- Difficult to measure patients with a very dark skin - False elevated levels after use of self-browning cream and sun blocker cream block
Eye lens autofluorescence	Eye lens	- Noninvasive - Fast technique (within seconds)	- Limited clinical research

AGEs, advanced glycation end products.





**Fig. 3.** Vascular effects of advanced glycation end products (AGEs). CAC, coronary artery calcium; FDG-PET,  $^{18}\text{F}$ -fluorodeoxyglucose-positron emission tomography; IMT, intima-media thickness; PWV; pulse wave velocity; RAGE, receptor for advanced glycation end products.

cells with AGE, which suggests that NF- $\kappa$ B is involved in this reaction [34]. Both TNF- $\alpha$  and ROS induce endothelial dysfunction and, therefore, stimulate the atherosclerotic process.

Besides these two pathogenic pathways, there is also evidence in support of local effects of AGEs on plaques in the arterial wall. AGE precursors are associated with macrophage apoptosis, causing rupture-prone atherosclerotic plaques [35]. In 75 carotid artery plaques, increased levels of MG-H1 and CML were found in histological identified rupture-prone plaques versus intermediate and stable plaques [35]. Furthermore, the latter study showed that the AGE precursor dicarbonyl methylglyoxal induced apoptosis of macrophages *in vitro*. Therefore, AGEs and their precursors are thought to be associated with unstable plaques, which in case of rupture provoke cardiovascular events.

The vascular effects of AGE are clinically relevant as shown by the associations between AGEs and atherosclerotic parameters, such as coronary artery calcium (CAC) score, intima-media thickness (IMT), and  $^{18}\text{F}$ -fluorodeoxyglucose-positron emission tomography (FDG-PET) (Fig. 3). In a cross-sectional study including 275 Japanese subjects, a positive association was found between glyceraldehyde-derived AGEs, measured with ELISA, and IMT, as well as between glyceraldehyde-derived AGEs and vascular inflammation, measured by FDG-PET [36]. Our research group has found that SAF is associated with IMT in 59 subjects without diabetes mellitus and cardiovascular disease, as well as with CAC score in patients with subclinical atherosclerosis ( $n = 67$ ) and PAD ( $n = 60$ ) versus controls ( $n = 96$ ) [37,38].

## 5. AGEs in PAD

As yet, six observational studies have been published in patients with PAD evaluating levels of AGEs measured in blood or with SAF (Table 2). Lapolla et al. were the first to show elevated serum AGEs including pentosidine in 33 type 2 diabetic patients with PAD versus 66 type 2 diabetic patients without PAD and 20 healthy control subjects [39]. All participants were evaluated by echo Doppler, and in patients with an ankle-brachial index (ABI)  $<0.90$ , PAD was confirmed by abnormal plethysmography. Serum AGEs were measured with ELISA and pentosidine was measured with liquid chromatography. In this study, ABI was inversely correlated with AGE and pentosidine in the diabetic patients. Obviously, these results were only correlations, and no multivariable models were performed in which could be corrected for cardiovascular risk factors. Furthermore, as mentioned above, results obtained from earlier available ELISA kits should be interpreted with caution.

Our research group found an association with SAF in a small subgroup of patients with both carotid artery stenosis and PAD versus carotid artery disease only [40]. This study was designed to detect the differences between patients with carotid artery stenosis and age- and sex-matched control subjects. Indeed, SAF was higher in patients with carotid artery stenosis. However, excluding the patients with PAD resulted in the loss of significant differences between carotid artery stenosis and control subjects. Determinants of SAF were age, smoking, diabetes mellitus, eGFR, and PAD. Lack of performing carotid duplex ultrasound examinations in control

**Table 2**

Overview studies performed in patients with peripheral artery disease.

Study	Type of AGE measurement	Design	Number of participants	Outcome	Data
Lapolla et al. [39]	Serum AGE + pentosidine	Case-control study	N = 33 PAD + DM N = 66 DM N = 20 controls	Highest serum AGEs and pentosidine in PAD	
Noordzij et al. [40]	SAF	Case-control study	N = 14 PAD + CAS N = 42 CAS N = 56 controls	Highest SAF in PAD	
Liu et al. [41]	SAF	Cross-sectional study	N = 94 PAD + DFU N = 24 DFU	SAF is associated with the presence of PAD	OR 5.98E <sup>9</sup> ; 95% CI 2.35E <sup>5</sup> –1.52E <sup>14</sup>
de Vos et al. [42]	SAF	Case-control study	N = 492 PAD N = 164 controls	SAF is associated with the presence of PAD	OR per unit increase of SAF 2.47; 95% CI 1.66–3.69
de Vos et al. [43]	SAF	Cohort study	N = 252	All-cause mortality and fatal and nonfatal MACE	HR per unit increase of SAF 1.63; 95% CI 1.13–2.34
de Vos et al. [44]	SAF	Cohort study	N = 252	Amputation	sHR per unit increase of SAF 1.50; 95% CI 1.04–2.17
Catalano et al. [45]	sRAGE	Case-control study	N = 201 PAD N = 201 controls	sRAGE is lower in PAD versus controls	2.72; 95% CI 1.38–5.39
Shiotsu et al. [46]	Ligand for RAGE S100A12	Case-control study	N = 26 PAD + ESRD N = 126 ESRD	S100A12 was associated with the presence of PAD	OR 5.71; 95% CI 1.29–25.30
Malmstedt et al. [47]	Ligand for RAGE S100A12 + RAGE score	Cohort study	N = 68	Major amputation or death	HR S100A12 above 75th percentile vs. below 2.58; 95% CI 1.05–6.35
Malmstedt et al. [47]	Ligand for RAGE S100A12 + CML + esRAGE	Case-control study	N = 68 PAD N = 30 controls	Highest levels of S100A12 and CML in PAD, while esRAGE was not different	HR per unit increase in RAGE score 2.23; 95% CI 0.85–5.88
Liu et al. [48]	sRAGE	RCT	N = 45 cilostazol N = 45 placebo	sRAGE significantly increased in the treatment group	

All odds ratios and hazard ratios represent the results of a multivariable analysis.

AGE, advanced glycation end products; DFU, diabetic foot ulcer; DM, diabetes mellitus; CML, N<sup>ε</sup>-carboxymethyl-lysine; esRAGE, endosecretory RAGE; ESRD, end-stage renal disease; HR, hazard ratio; OR, odds ratio; PAD, peripheral artery disease; RCT, randomized controlled trial; SAF, skin autofluorescence; sHR, subproportional hazard ratio; sRAGE, soluble receptor for AGE.

patients could explain the absence of a significant difference between the subgroup of carotid artery stenosis only versus control subjects, since the control subjects might be suffering from asymptomatic atherosclerotic disease. Neither the degree of stenosis nor differences between asymptomatic and symptomatic carotid stenosis were attributable to differences in SAF. Although the evidence for the association between PAD and SAF in this report is limited due to the low number of patients ( $n = 14$ , 25%), this study lends support to the hypothesis that SAF is associated with PAD.

Another cross-sectional study which showed an association between SAF and PAD in a subgroup was performed in Chinese patients with diabetic foot ulcers. A total of 94 (80%) of these patients had PAD [41]. In this study, the presence of PAD was positively and independently associated with age, glycated hemoglobin, and SAF. This study was designed to evaluate the role of SAF in patients with diabetic foot ulcers. Strong points of this study are that data of micro- and macrovascular complications were described extensively. Furthermore, multivariable analyses were performed for all micro- and macrovascular comorbidity individually. Unfortunately, this study was not designed as a case-control study with PAD patients. Therefore, this is underscored by the high number of patients with PAD. Therefore, this study is not appropriate for determining that SAF is increased in PAD compared to controls.

The largest cross-sectional study which evaluated the association between SAF and PAD was performed by our research group. A total of 492 patients with PAD was compared to age and diabetes mellitus matched control subjects ( $n = 164$ ) [42]. Strict inclusion and exclusion criteria were used. In case of an ABI < 0.90, the diagnosis of PAD was established using duplex ultrasound,

computed tomographic angiography, magnetic resonance angiography, or catheter angiography. Patients with end-stage renal disease (eGFR < 15 mL/min per 1.73 m<sup>2</sup>), recent myocardial infarction or stroke, and patients with an organ transplantation were excluded. SAF was higher in patient with PAD versus control subjects. In a logistic regression model including all patients, SAF was still significantly associated with PAD after adjustment for cardiovascular risk factors and comorbidity, with an odds ratio of 2.47 per unit increase of SAF; 95% confidence interval 1.66–3.69. Remarkably, SAF was not associated with ABI. This study was designed as a case-control study to evaluate whether SAF was increased compared to controls. Due to the strict inclusion and exclusion criteria, combined with the matching procedure and the multivariable regression analyses, this study was able to provide strong evidence for an increased SAF in patients with PAD compared to controls.

A total of 252 patients included in the latter study, could be followed for 5 years. Both cardiovascular endpoints as well as local outcome were evaluated. First, the endpoints all-cause mortality and fatal and nonfatal major adverse cardiovascular events (nonfatal MACE, defined as myocardial infarction and stroke) were analyzed. A total of 62 subjects (25%) died and fatal and nonfatal MACE occurred in 62 patients (25%) [43]. SAF was associated with all-cause mortality, also after adjustment for possible confounders (adjusted hazard ratio: 1.63 per unit increase of SAF; 95% confidence interval 1.13–2.34). SAF was also associated with fatal and nonfatal MACE, even after adjustment for confounders. A limitation in this study is that no blood, urine, or tissue was obtained from the patients at inclusion. Therefore, AGEs were only estimated with SAF and not directly measured in blood, urine, or tissue. Furthermore, no parameters for oxidative stress and inflammation were

available. Nevertheless, this study implies a role of SAF as a biomarker for cardiovascular events in patients with PAD, irrespective from risk factors such as age.

The second reported endpoint of local outcome was amputation during follow-up of 5 years. SAF was positively associated with amputation, analyzed with competing risk regression analysis with death as competing risk, subproportional hazard ratio from the multivariable analysis was 2.72; 95% confidence interval 1.38–5.39 [44]. In addition, a prediction model was tested for SAF. There was a positive interaction of SAF with Fontaine classification resulting in an area under the curve of 0.83; 95% confidence interval 0.74–0.92 for future amputation. A subgroup analysis was performed in patients with Fontaine I or II at inclusion. In this subgroup, SAF was the only predictor of amputation with a subproportional hazard ratio of 4.05; 95% confidence interval 2.09–7.83. SAF was not associated with intervention for PAD. This is the first model which demonstrates SAF as a useful marker in a prediction model for patients with PAD.

The last two studies are important because these are the first examples that SAF may have added value for risk differentiation in secondary cardiovascular risk prediction in patients with known PAD. These results correspond with previous studies in which SAF was shown to differentiate risk and thereby add clinically useful information for cardiovascular risk prediction in other high cardiovascular risk groups with type 2 diabetes or with renal failure [9,10]. The consequence of such a risk differentiation within a high risk group may be translated into targeted intensification of secondary preventive treatment.

## 6. RAGE in PAD

Another indirect approach to evaluate the AGEs-RAGE pathway is by measuring RAGE. Four research groups obtained information about soluble RAGE (sRAGE), endosecretory RAGE and S100A12, a ligand for RAGE in patients with PAD.

One study performed a case-control study and compared sRAGE in patients with PAD ( $n = 201$ ) versus controls ( $n = 201$ ). sRAGE, measured by ELISA, was significantly lower in patients with PAD versus control subjects [45]. sRAGE is supposed to operate as a decoy for circulating AGEs, thereby preventing the interaction of AGE with RAGE, and reducing inflammatory and oxidative stress reactions. A strength of this study is the selection of control subjects; all of them were screened for PAD with ABI measurement. A limitation of this study is the selection of only Caucasians without diabetes mellitus. Since diabetes mellitus is a strong risk factor for PAD, these patients do not reflect typical PAD patients. Furthermore, this study used an older ELISA kit, and therefore these results should be interpreted with care. Although a multivariable logistic regression analysis for the presence of PAD was performed, the results of the role of sRAGE with correction for risk factors was not shown.

Shiotsu et al. showed that PAD patients with end stage renal disease (ESRD) on hemodialysis had increased plasma levels of S100A12, a ligand for RAGE, versus ESRD patients without PAD [46]. Plasma S100A12 and ABI were associated with the occurrence of PAD. A limitation of this study is the low number of PAD patients ( $n = 26$ ). A plausible explanation might be due to the definition for diagnosis of PAD. In this study, PAD was defined by clinical symptoms or a history of PAD. Asymptomatic patients were therefore not included. Furthermore, the more easily obtainable ABI measurement was not used, because of the possibility of overestimation due to increased arterial media sclerosis in these patients.

Malmstedt et al. published a combined case-control and prospective cohort study. In the case-control study, plasma CML and S100A12 were elevated in PAD patients compared to age and sex-

matched healthy controls without cardiovascular disease or diabetes mellitus, while endosecretory RAGE, a scavenger for AGE which prevents AGE-RAGE interaction, was not increased in PAD patients [47]. No additional multivariable models were performed in the case-control study. In the prospective cohort study, S100A12 and a RAGE score, which combines plasma CML, S100A12 and sRAGE, were tested as a biomarker for the primary composed end point major amputation and death. Indeed, both S100A12, as well as the RAGE score, were associated with this end point, while only S100A12 remained significant after correction for cardiovascular risk factors [47]. A limitation of this multivariable regression model is that although adjustments were made for age and diabetes mellitus, other important risk markers for AGEs such as smoking and renal function were not included into the analysis. In addition, also AGE and CML was measured with immunohistochemical staining in venous tissue of PAD patients obtained from infringuinal bypass surgery.

In contrast to the observational studies on the relations with RAGE, Liu et al. performed an open randomized-controlled trial in PAD patients with diabetes mellitus. This study showed effects of the antiplatelet/antithrombotic agent, cilostazol, on ABI. After 52 weeks of active treatment ABI increased compared to placebo [48]. Plasma sRAGE was increased in the treatment group, but not in the placebo group. The increased sRAGE levels coincided with lower hsCRP, sVCAM and E-selectin levels in concert with the concept that sRAGE serve as a scavenger for circulating AGEs, thereby decreasing the detrimental effect of AGEs. In a multivariable linear regression analysis, sRAGE was the only significant determinant for change of ABI. A limitation of this study was that PAD only was determined with ABI, and no angiography or magnetic resonance angiography were used. Although this observation would suggest that sRAGE might have a role as marker of an intervention response, this observation needs confirmation in other intervention studies.

## 7. Therapeutic targets

In addition to the use of AGEs as a biomarker, lowering of AGEs might be a therapeutic target. Several studies were performed targeting reduction of AGE formation, as well as breakdown of existing AGEs. However, both, the AGE inhibitor, aminoguanidine, and the AGE breaker alagebrium were not safe or efficient enough to be introduced in clinical practice [49]. Furthermore, the study arm of the high dose RAGE inhibitor PF-04494700 was terminated due to adverse events (mental confusion and falls) in a randomized controlled trial in patients with Alzheimer disease [50]. The low-dose study arm resulted in a decreased decline of the cognitive function of the patients. These results should be interpreted with caution due to the high dropout and discontinuation of the study.

Although there are so far limited pharmacological possibilities to effectively reduce AGEs accumulation or their vascular effects, patients are able to influence their AGE levels by adjusting their lifestyle. As described earlier, tobacco increases the AGE accumulation to a serious degree. Furthermore, further studies are warranted to assess the effects of lowering dietary intake of AGEs by PAD patients. A randomized cross-over designed trial is currently being performed in non-diabetic adults with a 2-week period of high and low AGE diet [51].

## 8. Future perspectives

Before AGEs might be considered for adding predictive information to cardiovascular risk prediction, there are several steps to take. First, the association between SAF and cardiovascular endpoints are generated from one cohort study of patients with PAD. Therefore, these results should be repeated and confirmed in other



cohorts, and other research groups.

Second, the majority of the studies were performed with the use of the AGE Reader™. A case-control study with serum AGEs, measured with liquid chromatography, should identify whether the results from Lapolla et al. are reproducible [39]. Although the AGE Reader™ has been validated with skin biopsies, it would be of interest to know whether SAF correlates with AGEs in the arterial wall. It is likely that AGEs of arterial tissue and SAF correlate, since Hofmann et al. showed that SAF was strongly correlated to AGEs in cardiac tissue from patients with coronary artery disease [52].

Finally, the paucity of effective pharmaceutical or lifestyle interventions aimed at reducing AGE accumulation or effector pathways, limits the possibility for targeted intervention. As for the broader use of SAF or other AGE markers for risk prediction, more evidence is also needed that patients reclassified by SAF to higher cardiovascular risk, will benefit from intensified treatment. Especially in PAD patients considered at highest risk for amputation as determined by SAF, benefit from earlier surgical treatment should be ascertained in new studies.

## 9. Conclusion

Given the increasing prevalence of PAD worldwide, new biomarkers are required to better identify patients at highest risk. Several studies show a positive association between AGEs and PAD. The majority of the available studies were performed with the use of the AGE Reader™. SAF, as a measure for skin AGEs, was found to be useful to identify patient at highest risk for local and cardiovascular endpoints. Future studies are necessary to evaluate whether these high risk patients benefit from intensified treatment.

## Author contributions

LdV initially drafted this manuscript. All authors read, revised and approved the final version of the manuscript.

## Conflict of interest

Andries J. Smit is founder and shareholder of DiagnOptics Technologies BV, The Netherlands, manufacturing autofluorescence readers (<http://www.diagnoptics.com>). All other authors have nothing to disclose related to this paper.

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## References

- [1] F.G. Fowkes, D. Rudan, I. Rudan, V. Aboyans, J.O. Denenberg, M.M. McDermott, et al., Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis, *Lancet* 382 (2013) 1329–1340.
- [2] P.W. Wilson, R.B. D'Agostino, D. Levy, A.M. Belanger, H. Silbershatz, W.B. Kannel, Prediction of coronary heart disease using risk factor categories, *Circulation* 97 (18) (1998) 1837–1847.
- [3] R.M. Conroy, K. Pyorala, A.P. Fitzgerald, S. Sans, A. Menotti, G. De Backer, et al., Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project, *Eur. Heart J.* 24 (11) (2003) 987–1003.
- [4] A. Goldin, J.A. Beckman, A.M. Schmidt, M.A. Creager, Advanced glycation end products: sparking the development of diabetic vascular injury, *Circulation* 114 (6) (2006) 597–605.
- [5] H.L. Lutgers, R. Graaff, T.P. Links, L.J. Ubink-Veltmaat, H.J. Bilo, R.O. Gans, et al., Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes, *Diabetes Care* 29 (12) (2006) 2654–2659.
- [6] J.W. Hartog, A.P. de Vries, H.L. Lutgers, R. Meerwaldt, R.M. Huisman, W.J. van Son, et al., Accumulation of advanced glycation end products, measured as skin autofluorescence, in renal disease, *Ann. N. Y. Acad. Sci.* 1043 (2005) 299–307.
- [7] S. Krale, E. Zimmerer, M. Brueckmann, S. Lang, T. Kalsch, A. Rippert, et al., Elevation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in patients presenting with acute myocardial infarction, *Clin. Chem. Lab. Med.* 47 (4) (2009) 446–451.
- [8] T. Ikeda, K. Maruyama, N. Ito, A. Utagawa, M. Nagane, Y. Shiokawa, Serum pentosidine, an advanced glycation end product, indicates poor outcomes after acute ischemic stroke, *J. Stroke Cerebrovasc. Dis.* 21 (5) (2012) 386–390.
- [9] H.L. Lutgers, E.G. Gerrits, R. Graaff, T.P. Links, W.J. Sluiter, R.O. Gans, et al., Skin autofluorescence provides additional information to the UK Prospective Diabetes Study (UKPDS) risk score for the estimation of cardiovascular prognosis in type 2 diabetes mellitus, *Diabetologia* 52 (5) (2009) 789–797.
- [10] R. Meerwaldt, J.W. Hartog, R. Graaff, R.J. Huisman, T.P. Links, N.C. den Hollander, et al., Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients, *J. Am. Soc. Nephrol.* 16 (12) (2005) 3687–3693.
- [11] S. Jaisson, P. Gillery, Evaluation of nonenzymatic posttranslational modification-derived products as biomarkers of molecular aging of proteins, *Clin. Chem.* 56 (9) (2010) 1401–1412.
- [12] V.M. Monnier, D.R. Sell, Prevention and repair of protein damage by the Maillard reaction in vivo, *Rejuvenation Res.* 9 (2) (2006) 264–273.
- [13] C. Cerami, H. Founds, I. Nicholl, T. Mitsuhashi, D. Giordano, S. Vanpatten, et al., Tobacco smoke is a source of toxic reactive glycation products, *Proc. Natl. Acad. Sci. U. S. A.* 94 (25) (1997) 13915–13920.
- [14] T. Goldberg, W. Cai, M. Peppas, V. Dardaine, B.S. Baliga, J. Uribarri, et al., Advanced glycoxidation end products in commonly consumed foods, *J. Am. Diet. Assoc.* 104 (8) (2004) 1287–1291.
- [15] T. Koschinsky, C.J. He, T. Mitsuhashi, R. Bucala, C. Liu, C. Buening, et al., Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy, *Proc. Natl. Acad. Sci. U. S. A.* 94 (12) (1997) 6474–6479.
- [16] W. Cai, J.C. He, L. Zhu, M. Peppas, C. Lu, J. Uribarri, et al., High levels of dietary advanced glycation end products transform low-density lipoprotein into a potent redox-sensitive mitogen-activated protein kinase stimulant in diabetic patients, *Circulation* 110 (3) (2004) 285–291.
- [17] T. Miyata, Y. Ueda, A. Yoshida, S. Sugiyama, Y. Iida, M. Jadoul, et al., Clearance of pentosidine, an advanced glycation end product, by different modalities of renal replacement therapy, *Kidney Int.* 51 (3) (1997) 880–887.
- [18] E. Yagmur, F. Tacke, C. Weiss, B. Lahme, M.P. Manns, P. Kiefer, et al., Elevation of N(epsilon)-(carboxymethyl)lysine-modified advanced glycation end products in chronic liver disease is an indicator of liver cirrhosis, *Clin. Biochem.* 39 (1) (2006) 39–45.
- [19] K. Sebekova, V. Kupcova, R. Schinzel, A. Heidland, Markedly elevated levels of plasma advanced glycation end products in patients with liver cirrhosis - amelioration by liver transplantation, *J. Hepatol.* 36 (1) (2002) 66–71.
- [20] I. Giardino, D. Edelstein, M. Brownlee, Nonenzymatic glycosylation in vitro and in bovine endothelial cells alters basic fibroblast growth factor activity. A model for intracellular glycosylation in diabetes, *J. Clin. Invest.* 94 (1) (1994) 110–117.
- [21] N. Verzijl, J. DeGroot, S.R. Thorpe, R.A. Bank, J.N. Shaw, T.J. Lyons, et al., Effect of collagen turnover on the accumulation of advanced glycation end products, *J. Biol. Chem.* 275 (50) (2000) 39027–39031.
- [22] V.M. Monnier, V. Vishwanath, K.E. Frank, C.A. Elmetts, P. Dauchot, R.R. Kohn, Relation between complications of type I diabetes mellitus and collagen-linked fluorescence, *N. Engl. J. Med.* 314 (7) (1986) 403–408.
- [23] K.J. Welsh, M.S. Kirkman, D.B. Sacks, Role of glycated proteins in the diagnosis and management of diabetes: research gaps and future directions, *Diabetes Care* 39 (8) (2016) 1299–1306.
- [24] J.L. Scheijen, M.P. van de Waarenburg, C.D. Stehouwer, C.G. Schalkwijk, Measurement of pentosidine in human plasma protein by a single-column high-performance liquid chromatography method with fluorescence detection, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 877 (7) (2009) 610–614.
- [25] R. Meerwaldt, R. Graaff, P.H. Oomen, T.P. Links, J.J. Jager, N.L. Alderson, et al., Simple non-invasive assessment of advanced glycation endproduct accumulation, *Diabetologia* 47 (7) (2004) 1324–1330.
- [26] M.J. Noordzij, J.D. Lefrandt, R. Graaff, A.J. Smit, Dermal factors influencing measurement of skin autofluorescence, *Diabetes Technol. Ther.* 13 (2) (2011) 165–170.
- [27] D.R. Sell, V.M. Monnier, Molecular basis of arterial stiffening: role of glycation - a mini-review, *Gerontology* 58 (3) (2012) 227–237.
- [28] S.J. Zeman, D.A. Kass, Advanced glycation endproduct crosslinking in the cardiovascular system: potential therapeutic target for cardiovascular disease, *Drugs* 64 (5) (2004) 459–470.
- [29] M.G. van Eupen, M.T. Schram, T.T. van Sloten, J. Scheijen, S.J. Sep, C.J. van der Kallen, et al., Skin autofluorescence and pentosidine are associated with aortic stiffening: the maastricht study, *Hypertension* 68 (4) (2016) 956–963.
- [30] B. Hofmann, A.C. Adam, K. Jacobs, M. Rieme, C. Erbs, H. Bushnaq, et al., Advanced glycation end product associated skin autofluorescence: a mirror of vascular function? *Exp. Gerontol.* 48 (1) (2013) 38–44.
- [31] D.J. Campbell, J.B. Somaratne, A.J. Jenkins, D.L. Prior, M. Yui, J.F. Kenny, et al., Diastolic dysfunction of aging is independent of myocardial structure but associated with plasma advanced glycation end-product levels, *PLoS One* 7 (11) (2012) e49813.
- [32] S. Willemsen, J.W. Hartog, Y.M. Hummel, M.H. van Ruijven, I.C. van der Horst,

- D.J. van Veldhuisen, et al., Tissue advanced glycation end products are associated with diastolic function and aerobic exercise capacity in diabetic heart failure patients, *Eur. J. Heart Fail.* 13 (1) (2011) 76–82.
- [33] A. Bierhaus, S. Schiekofe, M. Schwaninger, M. Andrassy, P.M. Humpert, J. Chen, et al., Diabetes-associated sustained activation of the transcription factor nuclear factor-kappaB, *Diabetes* 50 (12) (2001) 2792–2808.
- [34] M. Morita, S. Yano, T. Yamaguchi, T. Sugimoto, Advanced glycation end products-induced reactive oxygen species generation is partly through NF-kappa B activation in human aortic endothelial cells, *J. Diabetes Complicat.* 27 (1) (2013) 11–15.
- [35] N.M. Hanssen, K. Wouters, M.S. Huijberts, M.J. Gijbels, J.C. Sluimer, J.L. Scheijen, et al., Higher levels of advanced glycation endproducts in human carotid atherosclerotic plaques are associated with a rupture-prone phenotype, *Eur. Heart J.* 35 (17) (2014) 1137–1146.
- [36] N. Tahara, S. Yamagishi, M. Takeuchi, A. Honda, A. Tahara, Y. Nitta, et al., Positive association between serum level of glyceraldehyde-derived advanced glycation end products and vascular inflammation evaluated by [(18)F]fluorodeoxyglucose positron emission tomography, *Diabetes Care* 35 (12) (2012) 2618–2625.
- [37] H.L. Lutgers, R. Graaff, R. de Vries, A.J. Smit, R.P. Dullaart, Carotid artery intima media thickness associates with skin autofluorescence in non-diabetic subjects without clinically manifest cardiovascular disease, *Eur. J. Clin. Invest.* 40 (9) (2010) 812–817.
- [38] M.A. den Dekker, M. Zwiers, E.R. van den Heuvel, L.C. de Vos, A.J. Smit, C.J. Zeebregts, et al., Skin autofluorescence, a non-invasive marker for AGE accumulation, is associated with the degree of atherosclerosis, *PLoS One* 8 (12) (2013 Dec 23) e83084.
- [39] A. Lapolla, F. Piarulli, G. Sartore, A. Ceriallo, E. Ragazzi, R. Reitano, et al., Advanced glycation end products and antioxidant status in type 2 diabetic patients with and without peripheral artery disease, *Diabetes Care* 30 (3) (2007) 670–676.
- [40] M.J. Noordzij, J.D. Lefrandt, E.A. Loeffen, B.R. Saleem, R. Meerwaldt, H.L. Lutgers, et al., Skin autofluorescence is increased in patients with carotid artery stenosis and peripheral artery disease, *Int. J. Cardiovasc. Imaging* 28 (2) (2012) 431–438.
- [41] C. Liu, L. Xu, H. Gao, J. Ye, Y. Huang, M. Wu, et al., The association between skin autofluorescence and vascular complications in Chinese patients with diabetic foot ulcer: an observational study done in Shanghai, *Int. J. Low. Extrem. Wounds* 14 (1) (2015) 28–36.
- [42] L.C. de Vos, M.J. Noordzij, D.J. Mulder, A.J. Smit, H.L. Lutgers, R.P. Dullaart, et al., Skin autofluorescence as a measure of advanced glycation end products deposition is elevated in peripheral artery disease, *Arterioscler. Thromb. Vasc. Biol.* 33 (1) (2013) 131–138.
- [43] L.C. de Vos, D.J. Mulder, A.J. Smit, R.P. Dullaart, N. Kleefstra, W.M. Lijfering, et al., Skin autofluorescence is associated with 5-year mortality and cardiovascular events in patients with peripheral artery disease, *Arterioscler. Thromb. Vasc. Biol.* 34 (2014) 933–938.
- [44] L.C. de Vos, J. Boersema, D.J. Mulder, A.J. Smit, C.J. Zeebregts, J.D. Lefrandt, Skin autofluorescence as a measure of advanced glycation end products deposition predicts 5-year amputation in patients with peripheral artery disease, *Arterioscler. Thromb. Vasc. Biol.* 35 (2015) 1532–1537.
- [45] M. Catalano, A. Cortelazzo, R. Santi, L. Contino, M. Demicheli, Y. Yilmaz, et al., The Pro12Ala polymorphism of peroxisome proliferator-activated receptor-gamma2 gene is associated with plasma levels of soluble RAGE (Receptor for Advanced Glycation Endproducts) and the presence of peripheral arterial disease, *Clin. Biochem.* 41 (12) (2008) 981–985.
- [46] Y. Shiotsu, Y. Mori, T. Hatta, N. Maki, K. Iida, E. Matsuoka, et al., Plasma S100A12 levels and peripheral arterial disease in end-stage renal disease, *Nephron Extra* 1 (1) (2011) 242–250.
- [47] J. Malmstedt, S. Frebelius, M. Lengquist, G. Jorneskog, J. Wang, J. Swedenborg, The receptor for advanced glycation end products (rage) and its ligands in plasma and infrainguinal bypass vein, *Eur. J. Vasc. Endovasc. Surg.* 51 (4) (2016) 579–586.
- [48] J.S. Liu, T.J. Chuang, J.H. Chen, C.H. Lee, C.H. Hsieh, T.K. Lin, et al., Cilostazol attenuates the severity of peripheral arterial occlusive disease in patients with type 2 diabetes: the role of plasma soluble receptor for advanced glycation end-products, *Endocrine* 49 (2015) 703–710.
- [49] L. Engelen, C.D. Stehouwer, C.G. Schalkwijk, Current therapeutic interventions in the glycation pathway: evidence from clinical studies, *Diabetes Obes. Metab.* 15 (8) (2013) 677–689.
- [50] D. Galasko, J. Bell, J.Y. Mancuso, J.W. Kupiec, M.N. Sabbagh, C. van Dyck, et al., Clinical trial of an inhibitor of RAGE-Abeta interactions in Alzheimer disease, *Neurology* 82 (17) (2014) 1536–1542.
- [51] B. de Courten, M.P. de Courten, C.G. Schalkwijk, K.Z. Walker, J. Forbes, Dietary advanced glycation end products consumption as a direct modulator of insulin sensitivity in overweight humans: a study protocol for a double-blind, randomized, two period cross-over trial, *JMIR Res. Protoc.* 4 (3) (2015) e93.
- [52] B. Hofmann, K. Jacobs, A. Navarrete Santos, A. Wienke, R.E. Silber, A. Simm, Relationship between cardiac tissue glycation and skin autofluorescence in patients with coronary artery disease, *Diabetes Metab.* 41 (2015) 410–415.